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RESULTS OF SEROLOGICAL EXAMINATIONS FOR TOXOPLASMOSIS IN DOMESTIC AND WILD ANIMALS IN CZECHOSLOVAKIA

[Following is a translation of an article by Otto Havlik and Jiri Hubner in the Czech-language periodical Ceskoslovenska epidemiologie, mikrobiologie a imunologie (Czechoslovak Epidemiology, Microbiology and Immunology), Prague, Vol. IX, No. 5/6, pages 391-397.]

In cases of infectious diseases in which the infection is transmitted to man through animals, one of the inseparable rates of epidemiology is to determine the morbidity of the sources of the infection. In anthropozoonoses, which are not as yet well known, the primary task is to determine the main infectious sources in relation to man and with regard to the possibilities for preventive measures.

Thus, in cases of toxoplasmosis, a disease already well studied from the viewpoint of human infection, we do not as yet know all the possible sources and reservoirs because toxoplasmosis has a cosmopolitan distribution, and in the various parts of the world various animals serve as the primary sources of infection. The question is complicated further by the fact that toxoplasmosis can be included also among the focal infections, which means that it exists in a circuit among wild animals in nature without direct relationship to man.

We still know very little about the incidence of toxoplasmosis in the central European animal populations, and even in our country, little attention has been given to this question. Because of this, in 1955 we began to carry out a serological survey of animals in the CSR (Ceskoslovenska Republika -- Czechoslovak Republic). At the beginning we examined various species of animals at random in order to find out which species can be important in the dissemination of toxoplasmosis (Havlik, O., Hubner, J.,) [See Note]. In the last two years we have concentrated on a systematic examination of all species which the pilot study showed to be of possible importance. In this paper we present the summary results of our examinations of domestic and free-living (i.e., wild) animals up to the present time. (Note: Cs. Epidemiol., Mikrobiol. a Imunol. VII-6: 396-402. 1958.)

In our study we were interested in answers to several questions. Primarily we wanted to find out the degree of infestation among the most abundant domestic and wild animals. Secondarily, we wanted to

determine, on the basis of these results. which species are most important as direct sources of human infection in our conditions. In this we also considered their living habits and relationships to man. In this connection we also had to find the answer to the question of specificity of the reaction used and of titer evaluation. Finally, we were interested in the problem of distribution of toxoplasmosis, i.e., whether and to what degree the disease circulates among the wild animals in our country.

A. Materials and Methods

Materials consisting of wild animals were acquired by trapping and hunting during our institute's field expeditions into the six provinces of Bohemia (Kraslice, Beroun, Litomysl, Hlinsko, Orlik, Slany). Material consisting of large domestic animals was also acquired during the field actions in the above mentioned provinces and from the Prague slaughter houses.

Table 1. Results of the examination of domestic animals for toxoplasmosis using the Sabin-Feldman reaction

	No. examined	No. negative	Titers										Total No. positive	Positive in %
			1:4	1:16	1:64	1:256	1:1024	1:4000	1:16,000	1:65,000	1:256,000	1:1,000,000		
Oryctolagus cuniculus rabbit	223	25	23	74	79	3	2	5	5	2	3	2	198	88 7
Felis domestica cat	215	51	20	73	53	13	4	1					164	76.2
Canis familiaris dog	161	78	15	14	18	25	9	2					83	51
Equus caballus horse	34	30	2	2									4	
Sus scrofa pig	188	139	25	14	4	6							49	26.0
Bos taurus beef cattle	338	255	62	15	6								83	24.5
Capra hircus goat	83	13	10	20	20	8	7	4	1				70	84.3
Ovis aries sheep	64	33	14	3	5	4	3	2					31	48.4
Total: 8 species	1,306	624	171	215	185	59	25	14	6	2	3	2	682	

Because the slaughter house slaughters animals from only one or two provinces each day, we were collecting only small numbers of blood samples per day in order to survey animals of various origins. Cats were supplied by the national enterprise Bioveta from various catchers primarily from central Bohemia and also directly from Prague. Rabbits were supplied mainly from 18 producers also from central Bohemia.

For the serological examination we have selected the Sabin-Feldman color test for the following reasons. First, we have had considerable experience with this reaction; second, we consider it reliable; and finally, the antibodies last for a long time. We believe that, analogous to other protozoan diseases, the immunity to toxoplasmosis has a non-sterile character and we therefore presume the presence of the parasite for the total period when a positive color test is obtained. From this presumption, a positive S-F reaction would be a direct indication of the presence of parasites and not just an indication of previous infection.

To insure true and reliable results, almost all of the sera were examined twice: first, we carried out a screening test and then serial dilutions.

B. Results

In all, we have examined serologically 2,212 animals: 1,306 domestic and 581 wild animals and 325 birds. Results are presented in tables.

Of the 1,306 domestic animals, we obtained 682 positive reactions, i.e., 52.2% if we consider even the lowest titers of 1:4. In titers of 1:16 and higher 511 sera reacted, i.e., 39.1%. The greatest morbidity was found in rabbits (89%, respectively 79%), goats (84%, respectively 72%), cats (76% respectively 67%) and dogs (51.5%, respectively 42.2%). In the case of dogs we must point out that the portion of the material used originated in the veterinary hospital, and therefore included sick animals.

Table 2. Results of examinations of wild animals for toxoplasmosis using the Sabin-Feldman reaction.

	No. examined	Negative	Titers						Total No. positive
			1:4	1:16	1:64	1:256	1:1024	1:4096	
Erinaceus europaeus roumanicus hedgehog	12	7	2	3					5
Chiroptera sp. (4 species) bats	31	31							
Lepus europaeus hare	83	59	7	13	4				24
Mus musculus domestic mouse	31	29	1				1		2
Apodemus sylvaticus	102	101	1						1
Rattus norvegicus rat	93	92			1				1
Clethr. glareolus	68	62	2	4					6
Microtus arvalis field mouse	102	102							
Microtus agrestis	16	11	3	2					5
Vulpes vulpes fox	4	3	1						1
Mustela nivalis ferret	11	9		1				1	2
Cervus elaphus stag	2	1			1				1
Capreolus capreolus deer	26	11	2	6	5	1	1		15
Total: 17 species	581	518	19	29	11	1	2	1	63

Of the 581 wild animals belonging to 17 species 63 were positive, i.e., 10.9%; in titers of 1:16 44 samples were positive, i.e., 7.6%. Positive titers were most frequent in deer (58%, titer of 1:16 and higher 50%) and hares (29%, respectively 20%). In contrast to this, only a small percentage (3.6%, respectively 1.9%) of small murine rodents was positive. Other wild mammals were examined only in small numbers and the data obtained cannot yet be evaluated.

Of the 325 birds belonging to 24 species, we obtained 17 positive reactions (5.2%, while only 5 had titers of 1:16 and higher, i.e., 1.6%). On the average, therefore, birds are only lightly infected. Positive titers (1:16 and higher) were found only in domestic geese (2 out of the 23 examined), pigeons (2 out of 37) and garden dove (1 out of 47). Other domestic birds (ducks, chickens) as well as 18 wild species were negative

C. Discussion

From the results presented, we can see that the positive results are quite frequent especially in some of the species. Positive results in more than one half of the domestic animals examined show that toxoplasmosis is spread among our animals to greater degree than previously believed.

If we want to answer the questions as to which animals are of the greatest importance in the transmission of the disease to man we must consider not only the degree of morbidity, but also the possibilities for the transmission of the infection and the living habits of the various species.

It is necessary to emphasize that we have examined healthy animals who showed no symptoms of the disease (except for a portion of the dogs) and that therefore, we were dealing with latent cases. This means that in all probability they are not passing the parasites at this time. However, we must consider the fact that these animals passed the parasites for a certain period of time after the infection during parasithemia. It is known that experimentally infected animals pass toxoplasma through the feces, urine, saliva and milk. Because of this, during parasithemia there is a great possibility of transmitting the infection by dirty hands or by inhaling the particles of the excreta, or through small skin abrasions.

This manner of transmission of the infection should be considered primarily in regard to those animals who live in close contact with man. These are mostly cats and dogs. Cats are the primary source of infection in our country because in contrast to other domestic animals, they live and sleep with humans in the same roon. The transmission of the infection when cuddling the cat or when scratched by it is easily possible

Another species also significantly involved in the transmission of toxoplasmosis to humans is the dog. Especially in foreign literature it is repeatedly designated as the primary source of infection. We have examined altogether 161 dogs, of which 80 were clinically ill (supplied by veterinary hospitals in Pardubice, Brno and Beske Budejovice regardless of diagnosis); of the remaining healthy dogs 59 were service dogs (German shepherds). The results were interesting. While 29% of the sick dogs reacted positively (in all titers) among the healthy ones 74% were positive (in the group of German shepherds 69.5%). This proves, we believe, that among the clinically-ill dogs toxoplasmosis had no role as an etiological agent, and that the morbidity among dogs is greatly flexible. However, the 51.5% of positive findings (respectively 42.2% in titers of 1:16 and higher) show that dogs can also be an important source of human infection in our country, although not as important as cats

Rabbits are extremely important Almost 90% morbidity and the fact that toxoplasmosis was found in all the rabbitries examined show that rabbits must be included among the main sources of the infection. The danger of rabbits is higher because they are eaten and the infection can be acquired during skinning and kitchen processing of latently-infected animals. It is not without interest that Janku [See Note], in his 1923 work which is considered a classic today, points out that it is interesting that a mother of a child with congenital toxoplasmosis raised many rabbits during her pregnancy and frequently ate their meat. (Note: Janku, J.: Cas. Lekices, 39-43, 1923.)

In a similar way hares are of great importance in the dissemination of toxoplasmosis. The fact that almost one-third of all the hares from various provinces examined were positive shows that toxoplasmosis is more frequent in the hare populations than has been suspected from the findings in dead hares. In relation to the fact that in CSR we shoot approximately 1,000,000 hares yearly, the hare must be considered to be yet another important source of human infection. Infection is obviously acquired, as from rabbits, during skinning and kitchen processing. Also the fact that more than one-half of the positive reactions obtained among deer indicates that this species can be also important in the dissemination of the infection, although not as drastically as hares. In no way can we diminish the importance of the animals if we consider that in CSR we shoot and consume more than 50,000 head annually.

A special chapter is taken up by the large domestic animals, primarily cows and pigs The total morbidity among these is also very high (25%), but if we consider the frequency of various titers we see an unusually frequent occurrence of the lowest titers of 1:4. In cows, pigs and sheep who were positive, full 62% of the titers were equal to 1:4. In the species mentioned above i.e., cat, dog, rabbit, hare and deer, the frequency of the titer

corresponds roughly to a normal (Gausse) curve and that of 1:4 titers equals 13.8% of the total positive cases. Similar situation has been determined by Feldman, Miller, Jones, Eyles and Gibson in serological examinations of animals in the USA. While the cat and goat titer frequency was regular, beef cattle and sheep exhibited unusually frequent cases of titers of 1:4 (49% of all positive titers).

The abnormally high number of the lowest titers can be explained in these animals by the fact that in many of them we are dealing with non-specific reaction due either to the influence of another infection which gives a cross-reaction at the lowest dilution, or due to the specific characteristics of the blood serum [See Note]. This question is still to be subjected, to detailed analysis before we can make a definite statement. Six percent of the positive cows and 13% of the pigs (titers of 1:16 and higher) indicate at the same time that these domestic animals are of no particular importance in the transmission of toxoplasmosis.

(Note: In our last work we considered the possibility of non-specific reaction in sarcocystic infection. Muhlpfordt, [See Note], Awad and Lainson [See Note] point out that animals infected with Sarcocystis tenella reacted positively. Awad actually elaborated a modification of the SF reaction while using spores of S. tenella. On the other hand, Cathie and Cecil (Lancet, 20:816, 1957) denied the statement of the above authors and proved that human and sheep sera contain a thermolabile factor which can influence the reaction but which has nothing to do with the SF antibodies. In the study of specificity of the SF reaction in our laboratory, we have carried out an experiment including the saturation of toxoplasmic sera with sarcocysts. Results showed that neither living sarcocystis nor dried antigen have any effect on the positive toxoplasmic serum, while a homologous antigen is always saturated (Zastera, Havlik [Note: Muhlpfordt, H: Z. Tropenmed. Parasit 3: 205-215, 1951. Awad, F. I., Laison, R: J. Clin. Path. 7: 152-156, 1954. Awad, F. I. Trans. roy Soc. trop. Med. Hyg. 48: 1954]). Because of this we believe that the cross-immunity between toxoplasmosis and sarcocystis does not exist.)

Weinmann and Chandler [See Note] in the USA point out pigs and pork meat as the probable source of the infection for man. They have proved experimentally that in toxoplasmosis the disease can circulate among rats and pigs as does trichinosis. Our findings do not show that in our country such a transmission is of large importance. Of the 93 rats examined, antibodies were found only in one, and even in pigs the morbidity is relatively low. Besides this, in our country pork is usually roasted or otherwise treated thermally (in contrast to other countries where meat is preferably eaten raw or semi-raw) so that the possibility of infection from pork is minimal. (Note: Weinman, D , Chandler, A H : Proc Soc Exp. Biol. [N.Y.] 87: 211-216, 1954.)

An exception to the large domestic animals are the goats who show up to 80% morbidity as well as regular titer frequency. This shows that goats can be an important factor in the spread of toxoplasmosis; the transmission is primarily through milk, which in our country is consumed in large quantities especially in the rural areas where it is also often used to feed other domestic animals.

Finally, it is necessary to mention our findings among wild animals. Positive reactions are much less frequent than in the domestic animals, with the exception of hares and deer. Undoubtedly these animals have little importance in the transmission of the infection to man primarily because humans come only rarely into a close contact with them. Exception is made by the synantropic rodents the house mouse and the rat; however, here only 2.4% respectively 1.6% can be designated as positive.

Even though these species are of little importance as sources of human infection, our findings are of some importance for the determination of the circulation of toxoplasmosis in nature. In this connection, it is worth mentioning that we also obtained positive serological reactions with hedgehogs, ferret, otter, Apodemus sylvaticus, Clethrionomys glareolus and Microtus agrestis, and therefore in animals among which until now toxoplasmosis had not been found. The findings of toxoplasmosis among wild animals, living without any direct relation to man in free nature, prove that toxoplasmosis belongs among the focal infections in the sense of the teachings of academician Pavlovsky To clarify the circulation of toxoplasmosis in nature will be the goal of our future investigations

In conclusions we would like to emphasize that our studies are not only important from the viewpoint of determining toxoplasmic sources, but also from the viewpoint of practical limitation of this infection. Because we have determined the most important species involved in the transmission of the infection to man, we are also presenting possibilities for preventing the infection. It is obvious, that, for example, pregnant women should avoid contact with cats and dogs and should not process rabbits and hares and should not drink unboiled milk.

D. Conclusions

1. The authors have carried out a serological examination of 2212 animals originating from various provinces of Bohemia using the color test of Sabin-Feldman. The results are presented in Tables 1 and 2.

2. Of the 1,306 domestic animals 682 were positive, i.e., 52.2%; in titers of 1:16 and higher 511 were positive i.e., 39.1%. The greatest morbidity was found in rabbits (89%, respectively 79%),

goats (81%, respectively 72%), cats (76%, respectively 67%) and dogs (51.5%, respectively 42.2%)

3. Of the 581 wild animals belonging to 17 species, 63 were positive (10.1%), in titers from 1:16 44 sera were positive (7.6%). Positive titers were most frequent in deer (58%, 50%, in titers of 1:16) and hares (29%, respectively 20%). In contrast to this, murine rodents showed only low morbidity (3.6%, respectively 1.9%).

4. Of the 325 birds only 17 were positive i.e., 5.2%, only five showed titers of 1:16 and higher (i.e., 1.6%). Higher titers were found only in domestic geese, pidgeons and a garden dove.

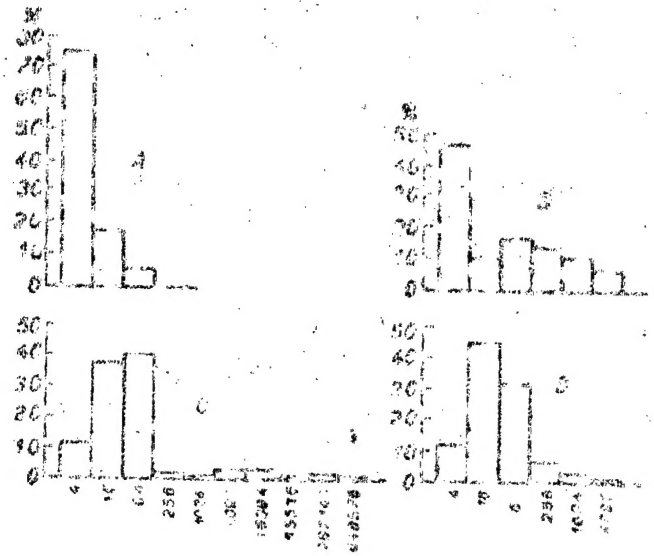
5. On the basis of the results obtained, the authors discuss the importance of the various species in the transmission of the infection to man. They consider cats the most important source of the infection and dogs the next most important source, primarily because of their close contact with man. Rabbits and hares are also of great importance, as infection can be acquired from them during kitchen processing.

6. It is not probable that in our country pigs and pork are important in disseminating the infection because the morbidity is low (only 13% had high titers). Rats are probably also not involved in the transmission in CSR

7. In the large domestic animals, namely cows, pigs and sheep, low titers have been found at a disproportionate rate. Titers of 1:4 form 62% of the positive titers. Among cats, dogs, rabbits, hares and deer the titers frequency corresponds roughly to the normal curve. 13.8% of the total count consists of titers of 1:4. The authors explain this by the fact that the high number of low titers in the first group of animals is caused by non-specific reactions (See Graph I.).

8. In conclusion, the authors recommend that as a preventive measure, pregnant women avoid contact with cats and dogs, avoid kitchen processing of rabbits and hares and refrain from drinking unboiled milk.

PICTURE APPENDIX



Frequency of positive titers of SF antibodies in some animals. A: cattle, B: sheep (example of an uneven spread of titers); C: rabbits, D: cats (regular distribution of titers).

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- END -